Ecological Entomology

Ecological Entomology (2020), DOI: 10.1111/een.12935

SHORT COMMUNICATION

Low-temperature physiology of climatically distinct south African populations of the biological control agent *Neochetina eichhorniae*

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Abstract. 1. *Neochetina eichhorniae* is the most widely established biocontrol agent on water hyacinth populations around South Africa. However, some *N. eichhorniae* populations have failed to adequately control their host population, specifically those exposed to cold conditions.

2. The aim of this study was to determine whether two climatically distinct populations of *N. eichhorniae* in South Africa differ in their low-temperature physiology, which tests whether local-climate adaptation has occurred.

3. We estimated weevil CT_{min}, LLT_{50}, SCP, and SCP mortality using standard approaches. Contrary to expectation based on climatic thermal profiles at the two sites, weevils from the warm locality ((mean ± SE) $CT_{min} = 5.0\,°C \pm 0.2$, $LLT_{50} = -11.3\,°C \pm 0.03$, SCP $= -15.8\,°C \pm 0.6$) were able to maintain activity and tolerate colder temperatures than the weevils from the colder site ($CT_{min} = 6.0\,°C \pm 0.5$, $LLT_{50} = -10.1\,°C \pm 0.1$, SCP $= -12.9\,°C \pm 0.8$).

4. These contradictory outcomes are likely explained by the poor nutrient quality of the plants at the cold site, driving low-temperature performance variation that overrode any macroclimate variation among sites. The cold site weevils may also have adapted to survive wide-temperature variability, rather than perform well under very cold conditions. In contrast, the mass-reared population of insects from the warm site has likely adapted to the consistent conditions that they experience over many years in confinement.

Key words. Climate change, cold adaptation, critical thermal limits, Curculionidae, water hyacinth.

Introduction

Water hyacinth, *Pontederia crassipes* (Martius) 1823 [≡*Eichhornia crassipes* (Martius) Solms-Laubach 1883] (Pontederiaceae), is South Africa's most damaging invasive aquatic weed (Hill, 2003). Biological control can be an efficient long-term management strategy, although its use within South Africa has been met with varied success (Hill, 2003).

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Neochetina eichhorniae Warner 1970 (Coleoptera: Curculionidae) is one of nine biocontrol agents released onto water hyacinth in South Africa (Hill & Coetzee, 2017). *N. eichhorniae* is a small weevil (4–5 mm), native to Argentina, that causes severe damage to *P. crassipes*, reducing the plant's ability to retain water and photosynthesise, and increasing susceptibility to pathogen infection (Julien, 2001). The weevil has, however, had difficulties establishing on a minority of *P. crassipes* infestations in South Africa, specifically those associated with colder conditions and eutrophic waters, which may occur in isolation, or congruently (Hill & Olckers, 2000; Jadhav *et al.*, 2007).

To better understand the relationship between thermal physiology and environmental conditions, various metrics of thermal performance are employed (Sinclair *et al.*, 2015). Key metrics

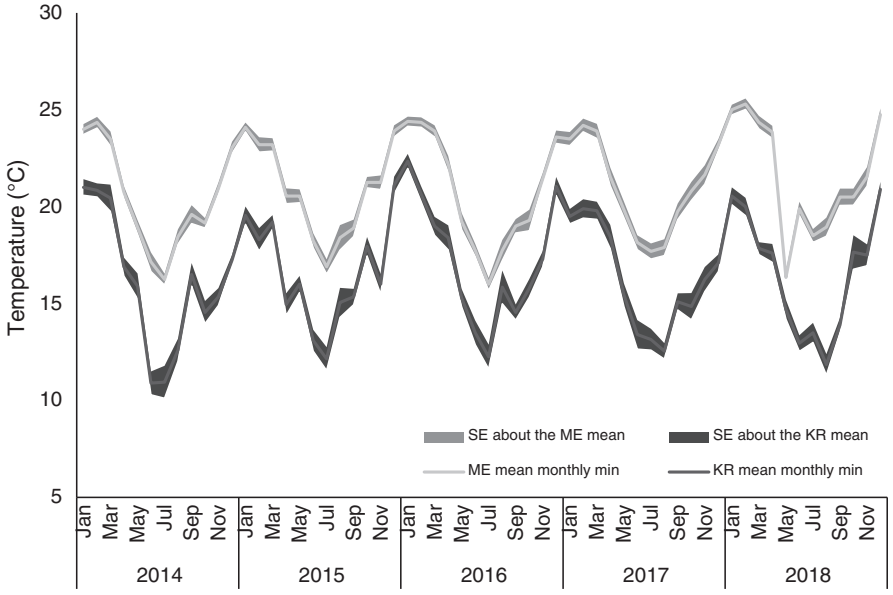

Fig. 1. Monthly mean minimum (min) temperatures calculated from daily minimum temperatures for Mount Edgecombe (ME) and for the DOHNE weather station, which represents the Kubusi River (KR) in this figure, between and including the years 2014 and 2018. The bands that surround the mean minimum temperature lines represent the SE of the mean.

include the critical thermal minima (CT_{min}), lower lethal temperature (LLT_{50}), and supercooling point (SCP). The CT_{min} is the temperature below which an organism's motor ability is impaired; the LLT_{50} is the temperature at which 50% of the organisms survive; and the SCP is the temperature at which an organism's extracellular fluids freeze, and is used to determine freeze tolerance strategy (Renault *et al.*, 2002).

Studies of thermal tolerances of other South African biocontrol agents have confirmed that local adaptation can occur (Griffith *et al.*, 2019; Porter *et al.*, 2019). For example, at the Kubusi River, another biocontrol agent of water hyacinth, *Eccritotarsus catarinensis* Carvalho 1948 (Hemiptera: Miridae) was shown to have significantly lower CT_{min} than a laboratory-maintained culture (Porter *et al.*, 2019). However, similar research has not been undertaken for *N. eichhorniae*. Thus, the aim here was to determine if the low-temperature physiology of *N. eichhorniae* differs across two sites that experience different climatic conditions (Fig. 1). From Griffith *et al.* (2019), we might anticipate that *N. eichhorniae* from the warmer site would have poor low-temperature tolerance relative to weevils from the colder site. However, the lack of biological control at the colder site suggested otherwise. We therefore entered this study without any firm expectation in comparing thermal limits of the two populations. We did, however, predict that these weevils would be freeze-intolerant based on previous literature on other South African weevils (Fraser *et al.*, 2017; Reddy *et al.*, 2019).

Materials and methods

Neochetina eichhorniae weevils were collected from two sites within South Africa. A field population of weevils was hand-collected from the Kubusi River (32.5031°S, 27.8802°E, Eastern Cape, South Africa), while mass-reared weevils were collected from the Nseleni River in Mount Edgecombe (28.6876°S, 31.9738°E, Kwa-Zulu Natal, South Africa) in 2007 and reared in the South African Sugarcane Research Institute (SASRI) (29.7222°S, 31.0507°E, Kwa-Zulu Natal, South Africa) without further introduction until these experiments. These two sites fall into different climatic regions (Fig. 1), with the Kubusi River in a temperate area with winter frosting (King, 2011), and Mount Edgecombe a comparably warm and stable climate year-round.

To confirm that the climates at the two sites differed significantly, daily average minimum temperatures between 2014 and 2018 were obtained from the South African Weather Service (SAWS) (Fig. 1). Temperature recordings were from the Dohne weather station (Eastern Cape, South Africa), which is the closest (±40 km) weather station to the Kubusi River, and the Mount Edgecombe weather station (KwaZulu-Natal, South Africa) (<1 km from SASRI). These temperature data provide a general estimate of the conditions experienced at both sites. To test whether any differences existed between the two sites, the number of days per year below the CT_{min} of the respective weevil population (as determined below) were calculated (Terblanche *et al.*, 2005). The monthly temperature variability was also determined by subtracting the monthly mean minima from the monthly mean maxima.

CT_{min}, LLT_{50}, SCP, and SCP mortality experiments ($n = 120$, $n = 320$, $n = 64$ and $n = 64$ respectively) were conducted using standard methods (Sinclair *et al.*, 2015). For all experiments, weevils were sexed, an equal ratio included, and the rate of temperature decrease was 0.25 °C/min. A factorial ANOVA was used to determine the fixed effects of 'population' and 'sex' and their interaction on the weevil's CT_{min}. A logistic regression (logit link function) was used to determine the LLT_{50} for the

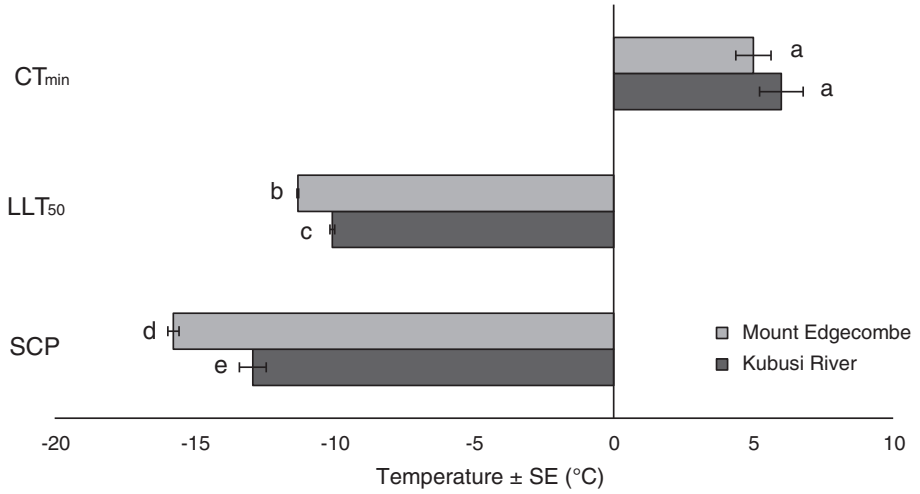

Fig. 2. The mean critical thermal minima (CT_{min}) ($n = 120$), lower lethal temperatures (LLT_{50}) ($n = 320$), and supercooling points (SCPs) ($n = 64$) of two populations of *Neochetina eichhorniae* from the Kubusi River and a mass rearing facility in Mount Edgecombe. Error bars represent SE and the letters 'a – e' denote significant differences between bars. As no significant differences were found between the sexes for any of the experiments, sex is not represented on the graph. '*n*' represents the sample size of total number of *Neochetina eichhorniae* weevils tested for the respective experiments.

weevils of both sexes and populations, and a factorial ANOVA was used to determine if 'population' and 'sex' had any influence on the LLT_{50}. The SCP, identified as the temperature recorded just prior to the latent heat of crystallization from individual insects, was visually determined. A General Linear Model was used to determine the influence of the variables 'population', 'mass' and 'sex' on the SCPs of the weevils. Key analyses assumptions were verified. Analyses were conducted using Statistica version 13 (TIBCO Software Inc.).

Results and Discussion

The mean minimum temperature from Dohne weather station dropped below the CT_{min} of the Kubusi River weevils an average of 65.46 ± 5.37 (SE) days each year between 2014 and 2018. At Mount Edgecombe, the mean minimum temperature only dropped below their CT_{min} an average of 0.42 ± 0.45 (SE) days per year for the same period. Thus, Kubusi River weevils are exposed to cold conditions for more days per year when compared to weevils from Mount Edgecombe. *Neochetina eichhorniae* are known to behaviourally counter adverse thermal conditions by huddling in-between petioles or in root masses where they are buffered to some extent from these conditions (DeLoach & Cordo, 1976). However, time spent huddling may be physiologically stressful, and since the congregated insects are not feeding on the plant, a comparison of days that drop below the insect's CT_{min} is a good measure of the differences between the two sites.

The CT_{min} of *N. eichhorniae* differed between populations [Kubusi River $CT_{min} = 6.00\,°C \pm 0.48$ (SE) $n = 60$, Mount Edgecombe $CT_{min} = 5.00\,°C \pm 0.20$ (SE) $n = 60$; $F_{1,116} = 7.30$, $P < 0.001$] (Fig. 2), but not sexes [Male $CT_{min} = 5.42\,°C \pm 0.33$ (SE) $n = 30$, Female $CT_{min} = 5.58\,°C \pm 0.34$ (SE) $n = 30$; $F_{1,116} = 0.20$, $P = 0.65$]. There were no interaction effects between sex and population ($F_{1,116} = 0.07$, $P = 0.79$). The CT_{min} of the Kubusi River population was much higher than that reported by Porter *et al.* (2019) [$CT_{min} = -0.3\,°C \pm 0.063$ (SE)] for *E. catarinensis* at the exact same site, yet comparable to the results of Griffith *et al.* (2019) for the latter species [from $4.8\,°C \pm 0.3$ (SE) in summer to $3.6\,°C \pm 0.3$ (SE) in winter]. Furthermore, it is slightly higher than the CT_{min} of $1.1\,°C \pm 0.2$ (SE) for Kubusi River *N. eichhorniae* recorded by Reddy *et al.* (2019). This highlights the need for multiple biocontrol agents at some sites, each with different low-temperature performance, but also that thermal performance can be variable within and among species. Some key external factors include season, site-specific climatic conditions and whether the population is field-based or mass-reared under more controlled conditions for extended periods of time.

LLT_{50} differed significantly between the populations [Kubusi River $LLT_{50} = -10.07\,°C \pm 0.09$ (SE) $n = 160$, Mount Edgecombe $LLT_{50} = -11.30\,°C \pm 0.03$ (SE) $n = 160$; Wald$\chi^2 = 13.35$, $P < 0.001$] (Fig. 2), sexes [Male $LLT_{50} = -15.00\,°C \pm 0.00$ (SE) $n = 80$, Female $LLT_{50} = -15.50\,°C \pm 0.00$ (SE) $n = 80$; Wald$\chi^2 = 29.02$, $P < 0.001$] and there was a signification interaction between population and sex (Wald$\chi^2 = 5.72$, $P = 0.02$). Compared to CT_{min}, here the Kubusi River *N. eichhorniae* were able to survive much lower temperatures than Kubusi River *E. catarinensis* reported in Griffith *et al.* (2019), where their LLT_{50} ranged between seasons from $-4.0\,°C$ to $-4.7\,°C \pm 0.1$ (SE).

There was no significant variation in SCP with mass or sex ($F_{1,52} = 0.03$, $P = 0.87$ and $F_{1,52} = 0.01$, $P = 95$, respectively) of *N. eichhorniae*. However, there was significant variation in SCP between the two populations of *N. eichhorniae* [Kubusi River SCP $= -12.9\,°C \pm 0.8$ (SE) $n = 32$, Mount Edgecombe SCP $= -15.8\,°C \pm 0.6$ (SE) $n = 32$; $F_{1,52} = 10.13$, $P < 0.001$] (Fig. 2). The SCP mortality experiment showed that none of the weevils from either population survived following the recovery period, and therefore that *N. eichhorniae* is freeze intolerant, a result that is in agreement with Reddy *et al.* (2019).

Two factors may have influenced the thermal physiology of the *N. eichhorniae* populations in this study. Firstly, climatic variability in a given location readily influences thermal traits and adaptation thereof (Arias *et al.*, 2011; Reddy *et al.*, 2019). Weevils subject to greater climate fluctuations may be under strong selection to increase thermal tolerance range. Indeed, this may explain the low-temperature responses of Kubusi River weevils, and through this survival is prioritised, rather than the ability to perform activity under cold conditions. Secondly, nutrient quality and food availability may also profoundly influence thermal physiology. Weevils from SASRI have access to high-quality fertilized plants year-round, and have had so for many years while being mass reared, while the Kubusi River water hyacinth is presumably of a lower nutrient quality. However, more importantly, the Kubusi River food source frosts over for a few weeks each year (King, 2011). Due to the presence of several concurrent stressors, the Kubusi River weevils may not be able to adapt low temperature behaviour, and survival is instead optimised. In summary, it appears that the long-term stability of abiotic and food quality conditions that the mass-reared insects have experienced may benefit them in terms of their cold tolerance when compared to a field population.

Because of its predictive and explanatory power, the thermal physiology of biocontrol agents requires more exploration. Incorporating thermo-physiological traits can help predict the performance of biocontrol agents, assess where populations might fail, and ultimately, enhance biocontrol programme efficacy.

Acknowledgements

Thanks to Rhodes University for funding and ethical clearance (# RU-DZE-2018-05-Q35). Weather data were provided by the South African Weather Service. We are grateful to the referees for constructive comments that helped improve the work. There are no conflicts of interest.

Author contribution

Candice Owen and John Terblanche were responsible for the design of the project. Daniel Rogers was responsible for the collection of data, as well as the initial write up. Candice Owen assisted in the statistical analysis and editing of the written work. John Terblanche assisted with editing the paper. All authors contributed to revision of the work and agreed on the final version.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Accepted 6 August 2020

Associate Editor: Hans Van Dyck